

AMENDMENTS TO THE SPECIFICATION

Please amend paragraphs [0012], [0041]-[0044], [00132], [00194] and [00196] as follows:

[0012] In one variation, the crystallization volume comprises the protein in a concentration between 1 mg/ml and 50 mg/ml; 5-50% w/v of precipitant wherein the precipitant comprises one or more members of the group consisting of PEG MME (polyethylene glycol monomethyl ether) having a molecular weight range between 300-10000, and PEG having a molecular weight range between 100-10000; optionally 0.05 to 0.5M additives wherein the additives comprises one or more members of the group consisting of sodium chloride, sodium tartrate, ammonium sulphate, sodium formate and lithium chloride; and wherein the crystallization volume has a pH between pH 4 and pH 8.

[0041] Figure 2 illustrates a crystal of the complex of ATP with AKT3-complex SEQ ID NO: 4.

[0042] Figure 3 lists a set of atomic structure coordinates for AKT3-SEQ ID NO: 4 as derived by X-ray crystallography from a crystal that comprises the protein. Reference numbers in column E of Figure 3 correspond to residue numbers of SEQ ID NO: 1. The following abbreviations are used in Figure 3: "X, Y, Z" crystallographically define the atomic position of the element measured; "B" is a thermal factor that measures movement of the atom around its atomic center; "Occ" is an occupancy factor that refers to the fraction of the molecules in which each atom occupies the position specified by the coordinates (a value of "1" indicates that each atom has the same conformation, i.e., the same position, in all molecules of the crystal).

[0043] Figure 4 illustrates a ribbon diagram overview of the structure of AKT3-SEQ ID NO: 4, highlighting the secondary structural elements of the protein.

[0044] Figure 5 illustrates the ATP binding site of ~~AKT3~~ SEQ ID NO: 4 based on the determined crystal structure for the molecule in the asymmetric unit corresponding to the coordinates shown in Figure 3.

[00132] Figure 5 illustrates the ATP binding site of ~~AKT3~~ SEQ ID NO: 4 based on the determined crystal structure for the molecule in the asymmetric unit corresponding to the structure coordinates shown in Figure 3. The catalytic site for ATP is located at the interface of the two lobes (Figure 5), however, the ATP ligand is not present in the crystal structure.

[00194] Recombinant proteins were isolated from E. coli DH10B-Tr1 cellular extracts by passage over Chitin (New England Biolabs) resin. The SSP intein-CBD tag was removed by overnight incubation at room temperature of the AKT3-bound resin in a pH7.0 buffer. It is noted that the cleavage reaction leaves 4 amino acids on the amino terminal of the AKT3 kinase domain (Cys-Arg-Ser-Leu, residues 227-230 of SEQ. ID No. 3, Figure 1). The amino acid sequence of the resulting protein is shown as SEQ. ID NO: 4 in Figure 1. The AKT3 kinase domain protein purity as determined on denaturing SDS-PAGE gel was 50%. It is noted that the purification process involved the use of egg-white lysozyme that co-eluted in a 50%/50% amount with the AKT3 kinase domain. The egg-white lysozyme was not removed from the AKT3 kinase domain protein preparation for the crystallization trials. AKT3 kinase domain was not phosphorylated during the isolation and purification procedures as confirmed by mass spectrometry. ~~AKT3~~ SEQ ID NO: 4 was concentrated to a final concentration of 25.9 mg/ml and stored at 4°C in a buffer containing 25mM TRIS-HCl pH 7.6, 250mM NaCl, 1mM EDTA and 5mM DTT.

[00196] AKT3 protein samples corresponding to SEQ ID NO: 4 were incubated with 2mM ATP and 4mM MgCl₂ before setting crystallization trials. Crystals were obtained after an extensive and broad screen of conditions, followed by optimization.